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(54) Title: DENDRITIC CELLS ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES

(57) Abstract: The present invention provides novel methods for immunotherapy. The invention provides immune cells and methods to generate them, with the capacity to at least in part reduce an immune response in a host. In one aspect the invention provides a method for generating a dendritic cell with the capacity to tolerise a T cell for antigen said T cell was specific for. comprising culturing peripheral blood monocytes from an individual to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said activated dendritic cell with said antigen T cell was specific for.

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Title: Dendritic cell activated in the presence of
glucocorticoid hormones are capable of suppressing antigen-
specific T cell responses.

The invention relates to the field of medicine. More in
particular the invention relates to the field of
immunotherapy.

5   **Background of the invention**

The remarkable immunostimulatory properties of DC reside in
their ability to transport antigens from peripheral tissues
to lymphoid organs where they present these antigens to T
10   cells in an optimal costimulatory context (1). To achieve
this complex sequence of events, DC exist in different
functional stages. Immature DC behave as sentinels in
peripheral tissues where they efficiently capture antigens.
Upon pathogen invasion, induction of protective T cell
15   responses requires the activation of immature DC into mature
immunostimulatory cells. DC activation is triggered in
inflamed tissues by cytokines such as IL-1 and TNF-a and by
bacterial components such as LPS (2, 3). Activated DC migrate
to T cell areas in the lymph nodes while upregulating their
20   costimulatory capacities and optimizing their antigen
presenting functions. Upon interaction with antigen-specific
T cells, DC activation is further completed through
engagement of the receptor-ligand (L) pair CD40-CD40L,
leading to the production of IL-12 (4, 5, 6), a key cytokine
25   for T helper (Th) type 1 and cytotoxic T lymphocyte (CTL)
priming (7).
       APC activation through CD40-CD40L interactions
represents an important immunoregulatory step for the
establishment of protective T cell immunity against pathogens
30   and tumors (8, 9, 10). This process also plays a key role in
the onset of destructive T cell-mediated disorders such as
auto-immune diseases, allograft rejection and graft versus
host disease (11, 12, 13). The current treatment of these

disorders largely relies on the administration of
glucocorticoids (GC), which exert potent anti-inflammatory
and immunosuppressive effects. Because GC negatively
interfere with many aspects of T cell activation such as IL-
5   2-driven proliferation and inflammatory cytokine production
(reviewed in 14), activated T cells have long been considered
as the main targets for GC action. Several lines of evidence
now suggest a role for DC in GC-induced immune suppression.
Moser et al (15) found that GC prevented the spontaneous
10   activation of murine DC thereby decreasing their T cell
stimulatory potential. Kitajima et al (16) showed that GC
could hamper the T cell-mediated activation of a murine DC
line. Viera et al reported that human DC exposed to GC were
poor producers of IL-12 upon LPS stimulation (17). These
15   findings only concern loss of typical DC features and
therefore favor a simple inhibitory role of GC on DC
activation. A more complex immunoregulatory action on the DC
system has not been considered.
The present invention resulted from a detailed analysis of
20   the impact of GC on the CD40-mediated activation of monocyte-
derived DC. These DC develop after culture with GM-CSF and
IL-4 (2, 18) or after transmigration through endothelial
cells (19) and are known to mature into the most potent human
Th1-type-inducing APC upon CD40 ligation (5, 20). Moreover,
25   these APC can easily be generated in large numbers and are
thereby the cells of choice for DC-based modulation of T cell
immunity (21, 22). In contrast to previous studies, the
present invention shows that GC such as dexamethasone (DEX)
do not merely prohibit DC activation, but that it converts
30   CD40 ligation on human monocyte-derived DC is transformed
into an alternative activation pathway. DEX profoundly affect
the CD40-dependent maturation of human monocyte-derived DC,
not only by preventing the upregulation of costimulatory,
adhesion and MHC surface molecules, but also by causing these
35   cells to secrete the anti-inflammatory mediator IL-10 instead
of the Th1 stimulatory cytokine IL-12. In agreement with

these phenotypic and functional changes, DC triggered through
CD40 in the presence of DEX are poor stimulators of Th1-type
responses. Most importantly, the present invention shows that
such DC are able to induce a state of hyporesponsiveness in
5   Th1 cells, indicating that these cells are capable of active
suppression of Th1-type immunity.

As already mentioned above, the impact of GC on DC has been
the subject of several previous studies by others. However,
10   in contrast with the present invention, these studies only
highlighted inhibitory effects of GC on the DC system. DEX
was found to block the upregulation of CD80, CD86 and MHC
class II molecules upon activation of murine spleen DC (15,
16), whereas very recently DEX was demonstrated to also
15   prevent the differentiation of DC from monocyte precursors
(28). In these studies, the inability of DC to acquire high
expression of costimulatory and MHC molecules was accompanied
with a decrease in their T cell stimulatory potential, but
the effect of GC on IL-12 production was not investigated. On
20   the other hand, Viera et al found that the effect of GC on
LPS-induced DC activation consisted in a 4-fold reduction of
IL-12p70 synthesis (17). This partial effect on IL-12
secretion contrasts with the complete suppression of IL-12p70
production which is subject of the present invention, and can
25   be explained by the fact that their GC-treated immature DC
were extensively washed prior to LPS stimulation. We indeed
found that upon removal of GC, the effects of these drugs on
immature DC were rapidly reversible. The continuous presence
of GC during CD40 triggering of DC was clearly preferred in
30   order to stably and completely modulate DC activation (data
not shown). Taken together, previous findings indicated that
the impact of GC on the DC system should be merely
interpreted as an inhibitory event. Importantly, the present
invention clearly demonstrates that GC such as DEX do not
35   simply suppress DC activation but rather redirect this
process towards a distinct functional program.

DC activation through engagement of CD40-CD40L is a key
stimulatory event for the generation of effective Th1 and
CD4-dependent CTL responses in vivo (10, 36, 37, 38). This
5   pathway however is also involved in the development of
unwanted T cell responses leading to autoimmune disease or
organ-transplant rejection (11, 12, 13). Until now, treatment
of patients suffering from such disorders largely relies on
the systemic administration of GC hormones. This treatment
10   does not only suppress pathogenic T cell responses but also
induces a general state of immunosuppression and metabolic
and endocrine side effects. The present invention
demonstrates that activation of human monocyte-derived DC
through CD40, in the presence of GC such as DEX, results in
15   an IL-10-producing APC that is a poor stimulator for Th1-type
responses and that can even confer hyporesponsiveness to Th1
cells. The present invention therefore indicates that such DC
loaded with appropriate antigens can be exploited as a novel
approach for specifically downregulating unwanted T cell
20   responses in vivo.

The dentritic cells of the invention posses different
capabilities than previously reported for dendritic cells.
25   One can therefore consider these cells to be part of a class
of cells distinct from the class formed by the "classical"
dendritic cells. The dendritic cells of the invention can be
used in a different way than the classical dendritic cells.
The dendritic cells of the invention can for instance be used
30   to suppress, at least in part, an undesired immune response
in a host. In one aspect invention therefore provides a
method for preparing a pharmaceutical composition for
reducing an unwanted T cell response in a host, comprising
culturing peripheral blood monocytes from said host to
35   differentiate into dendritic cells, activating said dendritic
cells in the presence of a glucocorticoid hormone and loading

said activated dendritic cells with an antigen against which
said T cell response is to be reduced. An unwanted T cell
response can be any type of T cell response. For instance,
but not limited to, a T cell response associated with an
5   auto-immune disease or a transplantation disease such as a
graft versus host disease or a host versus graft disease. A
pharmaceutical composition of the invention typically
comprises a dendritic cell of the invention suspended in a
liquid suitable for preserving the function of said dendritic
10   cell in said liquid and/or suitable for administration to a
host. A host preferably is a human. Preferably said host is
at risk of developing or is suffering from an auto-immune
disease or allergy. Preferably, said host suffers from or is
at risk of suffering from host versus graft disease and/or
15   graft versus host disease. With the term at risk is meant
that one expects that said host may develop said disease, for
instance but not limited to a host receiving a transplant.
Such a host is considered to at risk of developing host
versus graft disease. An antigen typically is a peptide
20   capable of binding to a major histocompatibility complex I
and/or II molecule. Such peptides are known in the art and a
person skilled in the art is capable of determining whether a
given peptide comprises an antigen or not. An antigen may be
derived from a naturally occurring protein. An antigen may
25   also be a synthetic peptide or equivalent thereof, preferably
with an amino-acid sequence equivalent to a peptide derived
from a protein.
In another aspect the invention provides a pharmaceutical
composition for reducing an unwanted T cell response in a
30   host, said composition being obtained by culturing peripheral
blood monocytes from said host to differentiate into
dendritic cells, activating said dendritic cells in the
presence of a glucocorticoid hormone and loading said
activated dendritic cells with an antigen against which said
35   T cell response is to be reduced. In one embodiment a method
is provided for reducing an unwanted T cell response in a

host, comprising administering a composition of the invention
to said host.
The invention further provides method for reducing an
unwanted T cell response in a host comprising culturing
5   peripheral blood monocytes from said host to differentiate
into dendritic cells, activating said dendritic cells and/or
their precursors in the presence of a glucocorticoid hormone
and loading said activated dendritic cells with an antigen
against which said T cell response is to be reduced and
10   administering said composition to said host.
In one embodiment of the invention said activation is done
through a CD40 receptor. Activation of DC through triggering
of the CD40 receptor can involve either incubation with a
CD8-CD40L fusion protein, a trimeric from of CD40L consisting
15   of CD40L-molecules to which a modified leucine zipper has
been attached, anti-CD40 antibodies, or cells that express
CD40L. Other signals that can be employed for the activation
of DC as described in the present invention include
lipopolysaccharide (LPS) and polyI/C.
20   In another aspect the invention provides a method for
obtaining an dendritic cell capable of tolerising a T-cell
for an antigen comprising providing said dendritic cell with
a glucocorticoid hormone, activating said dendritic cell and
providing said dendritic cell with said antigen. With the
25   term tolerising is meant that said dendritic cell has an
immunosuppressive effect on said T cell. A tolerised T cell
will essentially not respond with cell division when exposed
to a cell presenting an antigen said T cell would in the
untolerised state respond to with cell division. A tolerised
30   T cell will essentially not respond with killing a cell
presenting an antigen said T cell would in the untolerised
state respond to with cell kill.
In one embodiment said dendritic cell and/or a precursor
thereof is provided with said glucocorticoid hormone in
35   vitro. A T cell of the invention is preferably an antigen
specific T cell, preferably a cytotoxic T cell or a Th cell.

In another aspect the invention provides an isolated
dendritic cell capable of modifying the function of an
antigen specific Th cell ,which would otherwise enhance a
5   given immune response, resulting in a T cell that is capable
of reducing this immune response. In one embodiment the
invention provides a method for modifying an antigen specific
T-cell comprising providing an dendritic cell according to
the invention with said antigen and co-cultivating said T-
10   cell and said dendritic cell. Preferably, said co-cultivating
is performed in vitro. Said method may further comprise
multiplying said functionally modified T-cell.
The invention also provides an isolated funtionally modified
T-cell obtainable by a method according to the invention.
15   In another aspect the invention provides the use of a
glucocorticoid hormone for obtaining an dendritic cell
capable of functionally modifying a T-cell.
The invention also provides a pharmaceutical composition
comprising an dendritic cell and/or a functionally modified
20   T-cell according to the invention. The invention further
provides the use of a dendritic cell and/or a functionally
modified T-cell according the invention for the preparation
of a medicament.
The invention also provides a method for the treatment of an
25   individual suffering from or at risk of suffering from a
disease associated with at least part of the immune system of
said individual comprising providing said individual with an
dendritic cell and/or a functionally modified T-cell
according to the invention. Preferably, said dendritic cell
30   and/or said functionally modified T-cell, or precursors
thereof are derived from an HLA-matched donor. Preferably,
said HLA-matched donor is said individual.
Method of treatments of the invention are preferably use for
the treatment of an individual suffering from an auto-immune
35   disease, an allergy, a graft versus host disease and/or a
host versus graft disease.

Examples

Example 1

Impairment of CD40-CD40L-mediated phenotypic changes by DEX

We explored the impact of DEX on the phenotypic changes induced by CD40 ligation on immature monocyte-derived DC. In the absence of DEX, the fusion protein CD8-CD40L induced a strong upregulation of the costimulatory molecules CD80, CD86 and CD40, of the MHC class I and II molecules, of the adhesion markers CD54 and CD58 and of the DC maturation marker CD83 (Fig 1). In the presence of DEX, these CD8-CD40L-induced phenotypic changes were dramatically impaired: the upregulation of CD80, CD86, CD40, CD54, CD58 and of the MHC class I and II molecules was largely inhibited and CD83 was not expressed (Fig 1). Importantly, DEX-treated DC did not revert to a monocyte/macrophage stage as shown by the lack of expression of CD14 (Fig 1). Titration of DEX showed a complete inhibition of CD40-mediated phenotypic changes at 10^{-6} M and 10^{-7} M, a partial blockade at 10^{-8} M and no effect at 10^{-9} M and 10^{-10} (data not shown). In addition, DEX action was dependent on binding to the GC-receptor, since it was asbolished by simultaneous addition of the GC receptor antagonist RU486 (data not shown). In experiments performed with LPS or TNF-a as activation agents, similar results were obtained. However, the combination of DEX and TNF-alpha induced a massive cell death (viable cell recovery 5-10% of control cultures), a phenomenon that was not observed when DEX-treated DC were stimulated with LPS or through CD40 (viable cell recovery 60 to 100% of control cultures) (not shown).

We next analyzed whether activated DC could still be affected by DEX. DC incubated with CD8-CD40L for 48h and further exposed to DEX maintained a stable activated phenotype (Fig 2).

We conclude that DEX prevents the phenotypic changes induced by CD40 signals on immature DC and that already activated DC are resistant to DEX action.

5   Example 2
    DEX does not interfere with the regulation of DC antigen
    uptake machinery
    Unlike activated DC, immature DC efficiently internalize
    antigens through macropinocytosis and mannose receptor-
10 mediated endocytosis (2, 3, 25, 26). We analyzed whether DEX
    could affect the DC antigen capture machinery and its
    downregulation following CD40 cross-linking. As shown in Fig
    3, incorporation of FITC-BSA and FITC-mannosylated BSA by
    immature DC and by DEX-treated immature DC was comparable.
15 Upon CD40 triggering, a similar decrease of FITC-BSA and
    FITC-mannosylated BSA uptake by both DEX-treated and
    untreated DC was observed (Fig 3). These results were the
    first to indicate to us that DEX does not block all aspects
    of DC activation, since it does not interfere with the down-
20 regulation of the DC antigen capture machinery.

    Example 3
    DEX-treated CD40-triggered DC secrete IL-10 instead of IL-12
    A key feature of CD40-triggered DC for initiating T cell
25 immunity resides in their ability to produce the
    proinflammatory cytokine IL-12 (5, 6, 27). We investigated
    whether DEX affected IL-12 production by DC stimulated
    through CD40, and we explored the possibility that DEX could
    promote the secretion of the anti-inflammatory cytokine IL-
30 10. As shown in Fig 4, CD40 triggering of DC strongly induced
    IL-12p40 and IL-12p70 secretion (up to 120ng/ml and 170pg/ml
    respectively) but only poorly stimulated the production of
    IL-10 (up to 68pg/ml). In contrast, CD40 triggering of DEX-
    treated DC resulted in a dramatically reduced IL-12p40
35 production (up to 100 fold) and in the complete suppression
    of IL-12p70 secretion, whereas IL-10 production was strongly

enhanced (up to 50 fold) (Fig 4). Immature DC and their DEX-
treated counterparts failed to secrete detectable amounts of
IL-12 and IL-10 (Fig 4). Therefore, CD40 ligation of DC in
the presence of DEX triggers the secretion of high levels of
5   the anti-inflammatory cytokine IL-10 instead of IL-12.

     Example 4
     DEX-treated CD40-triggered DC are capable of suppressing Th1-
     type immunity
10   The strikingly modified response of DC to CD40 ligation
     in the presence of DEX prompted us to compare the T cell
     stimulatory potential of these cells with that of their DEX-
     untreated counterparts. In an allogeneic MLR, CD40-triggered
     DC induced a strong proliferative T cell response whereas the
15   addition of DEX prior to CD40 triggering reduced their T cell
     stimulatory capacity to that of immature DC (Fig 5). When
     tested for their ability to stimulate an hsp65-specific CD4^{+}
     Th1 clone, CD40-triggered DC pulsed with the hsp65 protein or
     with the specific peptide epitope p3-13 were found to be
20   potent inducers of both T cell proliferation and T-cell
     dependent IFN-g production (Fig 5). In contrast, in the
     presence of Ag-pulsed DEX-treated CD40-triggered DC, T cell
     proliferation and IFN-g production were significantly
     decreased (p<0.001 and p<0.01 respectively) (Fig 5). We next
25   investigated whether DEX-treated CD40-triggered DC were
     simply poor stimulators of Th1 cells, or whether they could
     exert suppressive effects on these T cells. We therefore
     tested hsp65-specific T cells stimulated with p3-13-pulsed
     DEX-treated CD40-triggered DC for their capacity to respond
30   to a second potent antigenic challenge. Fig 6 shows that
     preculturing T cells with CD40-triggered DC led to a strong T
     cell proliferation and IFN-gamma production upon second
     antigen-specific restimulation. In contrast, preculture with
     DEX-treated CD40-triggered DC resulted in a dramatically
35   reduced proliferative and IFN-gamma production capacity of
     Th1 cells. Thus, CD40 triggering of DC in the presence of DEX

results in APC that are not merely poor inducers of T cell
responses but that also induce a state of hyporesponsiveness
in Th1 cells.

5   Example 5
DEX-treated activated DC suppress anti-transplant immunity in
vivo

In a first attempt to test whether DEX-treated DC could
10   suppress unwanted immunity, a skin transplant model was set
up in mice. Skin transplants from C57Bl/6 mice, when grafted
onto fully allogeneic Balb/c recipient mice, are known to be
rapidly rejected as a result of a very strong allo-reactive
anti-transplant T cell immune response. Experiments were
15   performed to analyse whether pre-treatment of the recipient
mice with DEX-treated DC resulted in suppression of this host
versus graft response. We made use of an established murine
DC cell line of C57Bl/6 origin, named D1, which exhibits a
stable immature phenotype in vitro unless they receive an
20   activating signal through agonistic anti-CD40 antibodies or
LPS. This trigger results in fully matured DC that are highly
capable of CTL priming in vitro as well as in vivo (39;40).
Maturation of D1 involves strong upregulation of the cell
surface expression of the costimulatory molecule CD86 (B7.2),
25   the CD40-receptor and Class II MHC (fig. 7). Similar to our
observations in human DC, maturation of D1 in the presence of
DEX largely prevented the upregulation of these molecules at
the cell surface (Fig. 7). This suggested that the DEX-
treated D1 cells could be exploited to suppress T cell
30   immunity in vivo. Balb/c mice were therefore injected with D1
cells that had been activated in the absence or presence of
DEX. One week after immunisation, the allo-reactive response
of splenocytes of these mice, as well as of control mice, was
tested in vitro. Mice pre-treated with immature D1 cells that
35   had been cultured in the presence or absence of DEX did not
exhibit significantly altered Th1-type alloreactive immunity

as compared to control mice (Fig. 8). This is in accordance
with our observations with human DC, which also showed that
DEX-treated immature DC did not exhibit different properties
than normal immature DC. As expected, mice immunised with
5   fully matured D1 showed enhanced responsiveness. Importantly,
mice pre-treated with D1 activated in the presence of DEX
displayed greatly decreased Th1-type allo-reactive immunity
(fig. 8).

It was subsequently tested whether the reduction of allo-
10 responses in the latter group of mice, as measured in vitro,
would reflect decreased allo-reactive immunity in vivo.
Groups of Balb/c mice were immunized with D1 cells matured in
the absence or presence of DEX. Skin transplants of C57Bl/6
origin were grafted onto these mice one week later, and graft
15 survival was monitored. The grafts were rapidly rejected in
control mice and mice immunized with normally matured D1
cells (Fig. 9). Rejection was not enhanced in the latter
group, despite the stronger allo-reactive responses in these
mice (Fig. 8). Most likely, the response in the control mice
20 is already sufficient for maximal rejection. Importantly,
graft rejection is significantly ($p < 0.05$) delayed in mice
pre-treated with D1 matured in the presence of DEX (fig. 9).
This outcome corresponds with the greatly reduced allo-
reactivity in these mice as determined in vitro (Fig. 8).
25 In conclusion, the skin grafting experiments in mice
demonstrate that DC matured in the presence of DEX can indeed
be employed to suppress unwanted immunity such as against
transplants. Although in the experiments shown the
transplants are eventually rejected, it should be noted that
30 donor and recipient displayed large differences with respect
to transplantation antigens. In humans, donors and recipients
are matched as closely as possible for these antigens. Murine
experiments exploiting better matched donor and recipient
strains are underway. The data in Fig. 8 and 9 show that pre-
35 treatment with DEX-treated mature DC will result in much more
profound effects in a this setting.

Materials and Methods

5   *Generation of DC*

Immature DC were generated from peripheral blood monocyte precursors. Human PBMC from healthy donors, isolated through Ficoll-Hypaque density centrifugation were plated at 1.5×10^7 per well in 6-well plates (Costar Corp., Cambridge, MA) in

10 RPMI 1640 (Life Technologies, Paisley, Scotland) supplemented with 2mM glutamine, 100UI/ml penicillin and 10% FCS. After 2 h at 37^0C, the non adherent cells were removed and the adherent cells were cultured in medium containing 500U/ml IL-4 (Pepro Tech Inc. Rocky Hill, NJ) and 800U/ml GM-CSF (kindly

15 provided by Dr S. Osanto, LUMC, Leiden, NL) for a total of 7 days.

Activation of immature DC with a CD8-CD40L fusion protein

Activation of DC though CD40 was performed with a fusion

20 protein made of the extracellular domain of human CD40L and of the murine CD8a chain (CD8-CD40L). The CD8-CD40L cDNA described by Garrone et al (23) was transferred into an eukaryotic expression vector containing the hygromycin resistance gene, and used for the generation of stably

25 transfected Chinese Hamster Ovary (CHO) cells. Culture supernatants containing the CD8-CD40L fusion protein were concentrated with a pressurized stirred cell system (Amicon, Inc., Beverly, MA), checked for binding to CD40 and tested for optimal DC activation conditions (not shown). DC were

30 incubated at 5×10^5/ml/well in a 24-well plate (Costar Corp., Cambridge, MA) and activated in the presence of 1/10 CD8-CD40L supernatant. Cells and supernatants were analyzed after 48 h. Of note, control supernatants obtained from untransfected CHO cells or from CHO cells transfected with

the CD8a cDNA lacked DC activating functions and were similar
to culture medium.

DEX and RU486 treatment of DC

5   Seven days immature DC were treated with 10^{-6}M DEX (Sigma, St
Louis, MO) in the presence of GM-CSF and IL-4 or GM-CSF
alone. After 24 h, DC were analyzed or were further
stimulated via CD40 by adding the CD8-CD40L fusion protein to
the cultures as described above. In some experiments, the
10   glucocorticoid receptor antagonist RU485 (Roussel-UCLAF,
Romainville, France) was used at 10mM final concentration,
alone or in combination with DEX.

Analysis of DC surface phenotype by flow cyttometry

15   Cells were stained on ice with FITC or PE-conjugated mouse
monoclonal antibodies (MoAb) for 30 min in PBS 1% FCS and
were analyzed on a FACScan® (Becton Dickinson, San Jose, CA).
The following MoAb were used: FITC-anti-CD80 (BB1), PE-anti-
CD86 (FUN-1), FITC-anti-CD40 (5C3), PE-anti-CD54 (HA 58) and
20   PE-anti-CD58 (1C3) (Pharmingen, San Diego, CA), PE-anti-CD14
(L243) and PE-anti-HLA-DR (Mf-P9) (Becton Dickinson), PE-
anti-CD83 (HB15A) (Immunotech, Marseille, France) and PE-
anti-HLA class I (Tu 149) (Caltag Laboratories, Burlingame,
CA).

25

Antigen uptake experiments

DC were resuspended in medium buffered with 25mM Hepes. FITC-
BSA and FITC-mannosylated BSA (both from Sigma) were added at
1mg/ml final concentration and the cells were incubated at
30   37^{0}C, or at 0^{0}C to determine background uptake. After 1 h, DC
were washed extensively with iced-cold PBS and analyzed by
FACS® using propidium iodide to eliminate dead cells.

Cytokine detection by ELISA

Culture supernatants were analyzed in serial twofold
dilutions in duplicate. IL-12p70 was detected using a solid
phase sandwich ELISA kit (Diaclone Research, Besancon,
5   France) (sensitivity 3pg/ml). For IL-12p40 and IFN-g
detection, capture MoAb and polyclonal biotinylated detection
Ab were obtained from Peter van de Meijde (BPRC, Rijswijk,
NL) (sensitivity 10pg/ml). IL-10 was detected using the
Pelikine compact human IL-10 ELISA kit (CLB, Amsterdam, NL)
10   (sensitivity 3pg/ml).

Allogeneic mixed lymphocyte reaction (MLR)

Non adherent allogeneic adult PBMC from an unrelated
individual were cultured in 96-well flat-bottom plates
15   (Costar Corp., Cambridge, MA) at a density of 1.5×10^5/well
with various numbers of g-irradiated (3,000 rads) DC, in
triplicates. Proliferation was assessed on day 5 by
[^{3}H]thymidine uptake (0.5mCi/well, specific activity
5Ci/mMol, Amersham Life Science, Buckinghamshire, UK) during
20   a 16 h pulse.

Th1 stimulation assays

The Mycobacterium tuberculosis and M. leprae hsp65-specific,
HLA-DR3-restricted CD4+ Th1 clone Rp15 1-1 used in this study
25   recognizes an hsp65 determinant corresponding to peptide
residues 3 to 13 (p3-13) (24). HLA-DR-matched DEX-treated
immature DC and their DEX-untreated counterparts were pulsed
with 10mg/ml of p3-13 or with 10mg/ml of hsp65 for 2 h,
washed extensively and stimulated through CD40 as described
30   above. For Ag-pulsed DEX-treated immature DC, CD40 triggering
was performed in the presence of DEX. Hsp65 specific T cells
(10^4) were cultured with different numbers of g-irradiated
(3,000 rads) DC in 96-well flat-bottom plates (Costar Corp.)
in triplicates for 3 days. [^{3}H]thymidine incorporation was
35   measured on day 3 after a 16 h pulse. Before the addition of

[^{3}H]thymidine, 50ml of supernatants were collected from each
well and supernatants from triplicate wells were pooled to .
measure IFN-g production. To test hsp65-specific T cells
responsiveness to a second potent antigenic challenge, 10^4 T

5   cells were first cultured for 48h with 5×10^3 peptide-pulsed
DC prepared as above, then harvested and allowed to rest in
medium containing 5U/ml IL-2. Three days later, 10^4 viable T
cells were restimulated with 5×10^3 peptide-pulsed DC   .
generated from the same donor as used for the first culture

10   and tested for their ability to proliferate and to produce
IFN-g as previously described.

Statistical analysis
Covariance analysis was used to compare T cell proliferation

15   and IFN-g production as a function of DC number, between DEX-
treated CD40-triggered DC and DEX-untreated CD40-triggered DC
(Fig. 5).

Figure legends

Fig. 1 Pretreatment with DEX inhibits the phenotypic changes
induced by CD40 ligation.

5   Seven days immature DC were cultured for 24h in the absence
or the presence of 10^{-6}M DEX and activated via CD40 with the
CD8-CD40L fusion protein for 48h. The comparison with
immature DC maintained in medium alone is shown. Empty
histograms show the background staining with isotype controls

10   MoAb and solid histograms represent specific staining of the
indicated cell surface markers. Specific mean fluorescence
intensities are indicated. Mean fluorescence intensities of
isotype controls were between 3 and 4. Data are
representative of 4 independent experiments.

15

Fig. 2 DC triggered through CD40 maintain an activated
phenotype upon a subsequent DEX exposure.
Immature DC were activated with the CD8-CD40L fusion protein.
DEX (10^{-6}M) or medium control were added 48h later and cells

20   were analyzed after 2 additional days of culture. The
comparison with immature DC maintained in medium alone is
shown. Empty histograms show the background staining with
isotype controls MoAb and solid histograms represent specific
staining of the indicated cell surface markers. Specific mean

25   fluorescence intensities are indicated. Mean fluorescence
intensities of isotype controls were between 3 and 5. Data
are representative of 2 independent experiments.

Fig. 3 Pretreatment with DEX does not affect the regulation

30   of DC antigen uptake machinery.
Immature DC were incubated in the absence or the presence of
10^{-6}M DEX for 24h and further activated or not via CD40 with
the CD8-CD40L fusion protein for 48h. Cells were pulsed for
1h with medium containing either 1mg/ml FITC-BSA or 1mg/ml

35   FITC-mannosylated BSA. Empty histograms show the background

autofluorescence, Grey-filled histograms show the background
uptake at 0^0C and black-filled histograms show the specific
uptake at 37^0C. Data are representative of 3 independent
experiments.

5

Fig. 4 Pretreatment with DEX alters the cytokine secretion
profile of CD40-triggered DC.
DEX-exposed or control immature DC were left in culture
without further treatment or stimulated with the CD8-CD40L
10   fusion protein. Culture supernatants were harvested 48h later
and IL-10, IL-12p40 and IL-12p70 secretion were analyzed by
specific ELISA. Data are representative from 6 independent
experiments.

15   **Fig. 5** Pretreatment with DEX impairs the T cell stimulatory
capacities of DC activated via CD40 and leads to a state of
hyporesponsiveness of Th1 cells.
Allogeneic MLR: non adherent allogeneic PBMC were cultured
with different numbers of CD40-triggered DC, DEX-treated
20   CD40-triggered DC or immature DC. The proliferative response
was measured on day 5.
Th1 stimulation assays: Hsp65-specific T cells were cultured
with different numbers of HLA-DR matched CD40-triggered DC or
with DEX-treated CD40-triggered DC pulsed with the hsp65
25   protein or with the specific p3-13 peptide epitope. The
proliferative response and the T cell dependent IFN-g
production were analyzed on day 3. Data are representative of
4 independent experiments.

30   **Fig. 6** DEX-treated DC triggered through CD40 induce a state
of hyporesponsiveness in Th1 cells. Hsp65-specific T cells
precultured with CD40-triggered DC or with DEX-treated CD40-
triggered DC pulsed with the p3-13 peptide epitope were
harvested after 48h, allowed to rest in the presence of 5U/ml
35   IL2 for 3 days, and restimulated with p3-13-pulsed DC. The

proliferative response and IFN-g production were measured on
day 3. Similar results were obtained in 2 independent
experiments.

5   **Fig. 7** D1 cells were left untreated or cultured for two days
     in the presence of LPS (1 ug/ml) and/or DEX (10-6 M). Surface
     expression of CD86 (B7.2), Class II MHC and CD40 was
     monitored by staining with the appropriate FITC-labeled Ab.
     and subsequent FACS-analysis. Similar results were obtained
10   for D1 cells matured in the presence of murine CD40L (not
     shown).

     Fig. 8 Balb/c mice were immunised with 10-6 D1 cells that
     were prepared as indicated. One week later, splenocytes were
15   isolated and tested for their response against C57Bl/6
     splenocytes in a mixed lymphocyte culture. After 24 h,
     culture supernatants were tested for IFNg content in a
     specific ELISA assay.

20   **Fig. 9** Balb/c mice (5 per group) were immunised as indicated.
     One week later, skin transplants of C57Bl/6 origin were
     grafted and graft survival was monitored.

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acquire CD8(+) cytotoxic T lymphocyte priming capacity
upon activation by T helper cell-independent or -dependent
stimuli. J Exp Med 2000 Jul 3;192(1):145-50

CLAIMS

1.   A method for preparing a pharmaceutical composition for reducing an unwanted T cell response in a host, comprising culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic
5   cells in the presence of a glucocorticoid hormone and loading said dendritic cells with an antigen against which said T cell response is to be reduced.

2.   A pharmaceutical composition for reducing an unwanted T cell response in a host, said composition being obtained by
10 culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said dendritic cells with an antigen against which said T cell response is to be reduced.

15 3.   A method for reducing an unwanted T cell response in a host, comprising administering a composition of claim 2 to said host.

4.   A method for reducing an unwanted T cell response in a host comprising culturing peripheral blood monocytes from
20 said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said dendritic cells with an antigen against which said T cell response is to be reduced and administering said composition to said host.

25 5.   A method according to claim 1,3 or 4 whereby said activation is done through a CD40 receptor.

6.   A method according to claim 5 whereby said activation involves incubation of the dendritic cells with either CD8-CD40L fusion protein, a trimeric from of CD40L consisting of
30 CD40L-molecules to which a modified leucine zipper has been attached, anti-CD40 antibodies, or cells that express CD40L.

7.   A method according to claim 5 whereby said activation involves incubation of the dendritic cells with lipopolysaccharide (LPS) or polyI/C.

8.   A method according to claim 1, 3-7 whereby said
dendritic cells are infected with one or more recombinant
viruses encoding the antigen(s) of interest before activating
said dendritic cells in the presence of a glucocorticoid
5   hormone.

9.   A method according to claim 1, 3-8 whereby said
dendritic cells are incubated with one or more recombinant
proteins or large (> 20 amino acids) synthetic peptides
representing the antigen(s) of interest before activating
10   said dendritic cells in the presence of a glucocorticoid
hormone.

10. A method according to claim 1,3 or 9 whereby said
dendritic cells are incubated with cells or cell homogenate
containing the antigen(s) of interest before activating said
15   dendritic cells in the presence of a glucocorticoid hormone.

11. A method according to claim 1, 3-10 whereby said
dendritic cells are loaded with synthetic peptides
representing the antigen(s) of interest after activating said
dendritic cells in the presence of a glucocorticoid hormone.

20   12. A method according to claim 1, 3-11 whereby said
dendritic cells, after activation in the presence of a
glucocorticoid hormone, secrete interleukin-10.

13. A method for obtaining a dendritic cell capable of
tolerising a T-cell for an antigen comprising providing said
25   dendritic cell with a glucocorticoid hormone, activating said
dendritic cell and providing said dendritic cell with said
antigen.

14. A method according to anyone of claims 1, 3-13, wherein
said dendritic cell and/or a precursor thereof is provided
30   with said glucocorticoid hormone in vitro.

15. A method according to anyone of claims 1, 3-14, wherein
said T-cell is a T-helper cell.

16. An isolated dendritic cell prepared according to anyone
of claims 1, 3-15 capable of functionally modifying an
35   antigen-specific T-cell with respect to the response to said
antigen.

17. A method for functionally modifying an antigen-specific
T-cell comprising providing an dendritic cell according to
claim 16 with said antigen and co-cultivating said T-cell and
said dendritic cell.

5   18. A method according to claim 17, wherein said co-
cultivating is performed in vitro.
19. A method according to claim 17 or claim 18, further
comprising multiplying said functionally modified T-cell.
20. An isolated functionally modified T-cell obtainable by a

10   method according to anyone of claims 17-19 that is capable,
upon administration to the host, of reducing an unwanted
immune response.
21. Use of a glucocorticoid hormone for obtaining an
dendritic cell capable of functionally modifying a T-cell.

15   22. A pharmaceutical composition comprising an dendritic
cell according to claim 16 and/or a functionally modified
T-cell according to claim 20.
23. Use of an dendritic cell according to claim 16 and/or a
functionally modified T-cell according to claim 20 for the

20   preparation of a medicament.
24. A method for the treatment of an individual suffering
from or at risk of suffering from a disease associated with
at least part of the immune system of said individual
comprising providing said individual with an dendritic cell

25   according to claim 16 and/or a functionally modified T-cell
according to claim 20.
25. A method according to claim 24, wherein said dendritic
cell and or said T-cell is derived from an HLA-matched donor.
26. A method according to claim 24 or claim 25, wherein said

30   dendritic cell and or said T-cell is derived from said
individual.
27. Use of an dendritic cell according to claim 16 in a
treatment for an individual suffering from an auto-immune
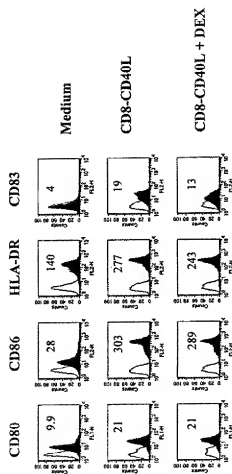disease, allergy, a graft versus host disease and/or a host

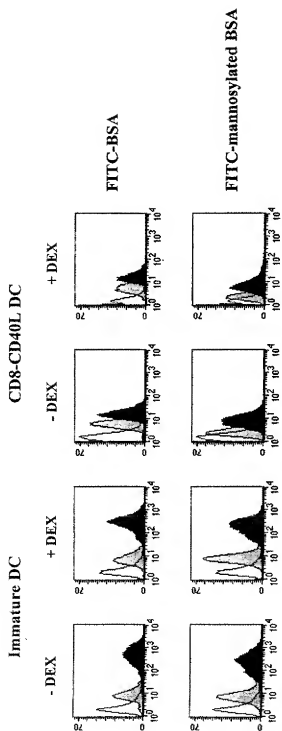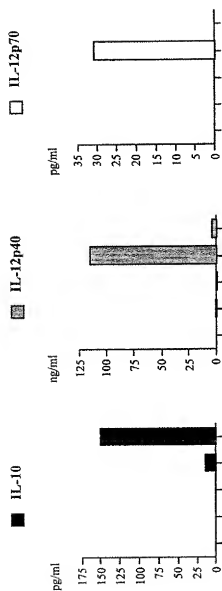35   versus graft disease.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

T cells initially cultured with:

☐ p3-13 pulsed CD8-CD40L DC

■ p3-13 pulsed DEX + CD8-CD40L DC

IFN-γ production

pg/ml

2000—
1500—
1000—
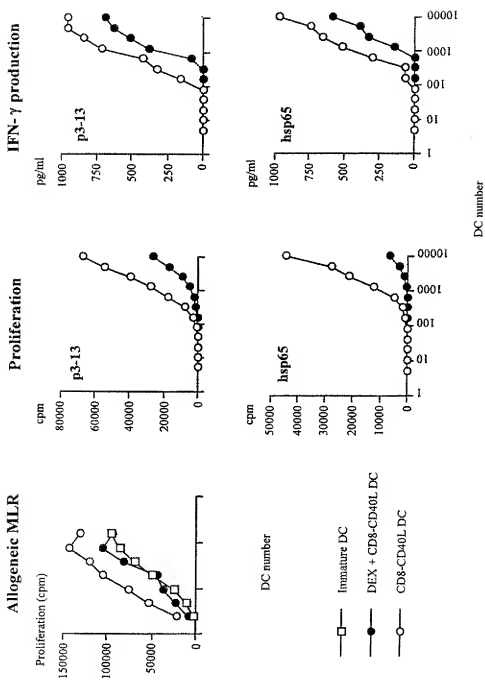500—
0—

Proliferation

cpm
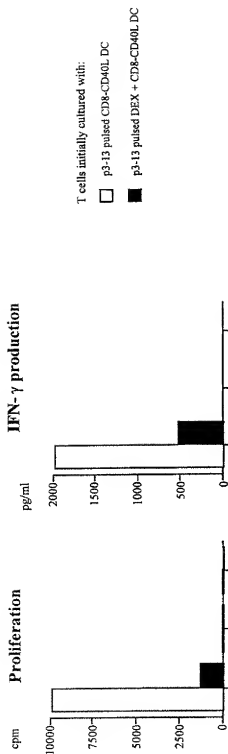
10000—
7500—
5000—
2500—
0—
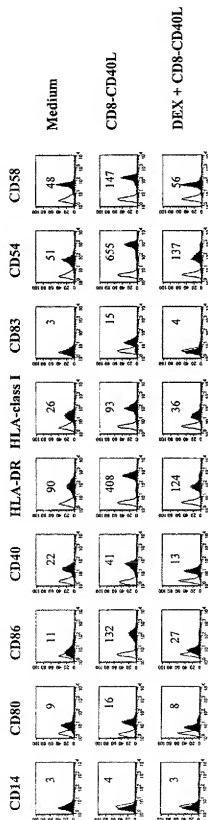
Fig. 6

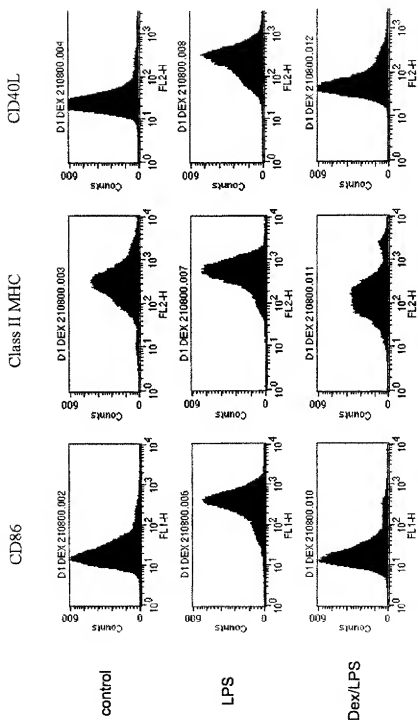Fig. 7. DEX modulates LPS-induced maturation of murine DC

Figure 8. Decreased allo-reactivity after pre-treatment with DEX-DC

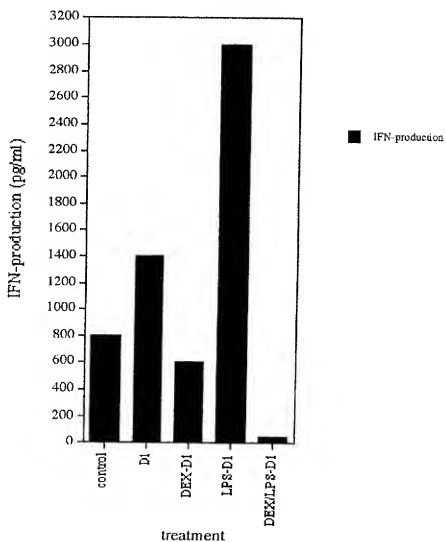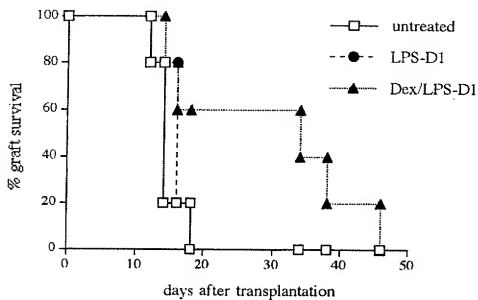Figure 9. Prolonged graft survival after pre-treatment with DEX-DC

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7   A61K39/00   A61K35/14   C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7   A61K   C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VIEIRA P L ET AL: "Glucocorticoids inhibit bioactive IL-12p70 production by in vitro-generated human dendritic cells without affecting their T cell stimulatory potential." JOURNAL OF IMMUNOLOGY, vol. 161, no. 10, 15 November 1998 (1998-11-15), pages 5245-5251, XP000973668 cited in the application	1-7, 9-16, 22-27
Y	the whole document	8-12, 14-16, 22-27

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☒ Further documents are listed in the continuation of box C.   ☐ Patent family members are listed in annex.

Date of the actual completion of the international search	Date of mailing of the international search report
7 February 2001	02. 03. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Bilang, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	LU L ET AL: "Adenoviral delivery of CTLA4Ig into myeloid dendritic cells promotes their in vitro tolerogenicity and survival in allogeneic recipients" GENE THERAPY,MACMILLAN PRESS LTD., BASINGSTOKE,GB, vol. 6, no. 4, 1999, pages 554-563, XP000946114 ISSN: 0969-7128 the whole document	8-12, 14-16, 22-27
A	PIEMONTI L ET AL: "Glucocorticoids affect human dendritic cell differentiation and maturation." JOURNAL OF IMMUNOLOGY, vol. 162, no. 11, 1 June 1999 (1999-06-01), pages 6473-6481, XP002159692 cited in the application the whole document	1-27
A	GAO J X ET AL: "CD40-deficient dendritic cells producing interleukin-10, but not interleukin-12, induce T-cell hyporesponsiveness in vitro and prevent acute allograft rejection." IMMUNOLOGY, vol. 98, no. 2, October 1999 (1999-10), pages 159-170, XP000973593 the whole document	1-27
A	CHAKRABORTY ABALOKITA ET AL: "Stimulatory and inhibitory maturation of human macrophage-derived dendritic cells." PATHOBIOLOGY, vol. 67, no. 5-6, March 2000 (2000-03), pages 282-286, XP002159693 ISSN: 1015-2008 the whole document	1-27
P,X	REA D ET AL: "Glucocorticoids transform CD40-triggering of dendritic cells into an alternative activation pathway resulting in antigen-presenting cells that secrete IL-10." BLOOD , vol. 95, no. 10, 15 May 2000 (2000-05-15), pages 3162-3167, XP002159694 the whole document	1-27

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INTERNATIONAL SEARCH REPORT

Box I	Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   > Although claim(s) 3-12, 14, 16, 17, 19, 24, and 27 are directed to a
   > diagnostic method practised on the human/animal body or treatment of the
   > human/animal body, the search has been carried out and based on the alleged
   > effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all
   searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report
   covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.